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Establishing a relationship between semen evaluation parameters of freezable and non-freezable Murrah buffalo bull semen

YOGITA DHAKA[1], MADHUMEET SINGH[1], AKSHAY SHARMA[1]✉ and PRAVESH KUMAR[1]

Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh 176 062 India

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ABSTRACT

The present study was conducted with an objective of establishing a relationship between semen evaluation parameters of freezable and non-freezable Murrah buffalo semen. Work was conducted at Frozen Semen Bank, RCDF Limited, Bassi, Jaipur, Rajasthan, on 12 apparently healthy pure bred Murrah buffalo breeding bulls. A total of 96 ejaculates (48 each freezable and non-freezable) were analysed at five different stages of processing namely neat semen evaluation, post dilution, post equilibration, post thaw and after 1 h of incubation post-thaw at 37°C for progressive motility, live dead count, reaction to hypo-osmotic solution and acrosomal integrity. Evaluation of the semen was done in two groups, i.e. freezable and non-freezable semen. There was a significant difference in terms of all the semen evaluation parameters between freezable and non-freezable semen at different stages of evaluation. Also, results of the study revealed a highly significant correlation between the various semen evaluation parameters for both the groups.

Keywords: Acrosome, Hypo-osmotic swelling test, Murrah bulls semen, Progressive motility, Viability

Buffalo, through their potential for producing milk, meat and draft power, contribute significantly to the agricultural economy of many developing countries including India. Artificial insemination (AI) in buffaloes on large scale using semen from bulls with superior germplasm can solve the problem of low productivity as well as reproductive efficiency. During last three decades, extensive research work has been carried out both in India and abroad on various aspects for improving the freezing technology of buffalo bull semen. In spite of this improvement, the post-thaw semen of buffalo bulls is not as freezable as that of cattle (Dhami *et al*. 1995). The viability and fertility of frozen–thawed buffalo bull spermatozoa is considerably lower as these are more susceptible to hazards during freezing and thawing than cattle spermatozoa which create hurdles in extensive exploitation of production potential of buffalo. Therefore, a better understanding of the fundamental principle of cryopreservation of buffalo spermatozoa is necessary as per its specific requirements.

One of the many possible causes of lower freezability of buffalo bull semen compared to cattle bull can be due to the differences in the lipid ratio of the spermatozoa (Andrabi 2008). Freezing-thawing of buffalo spermatozoa is known to cause considerable damage to DNA, motility apparatus, plasma membrane and acrosomal cap (Rasul *et al.* 2001), leakage of intracellular enzymes (Dhami and Kodagali 1990) and thus, reduced fertility. Although some of the bulls may apparently donate freezable semen but the freezability may be non-freezable adversely affecting fertility, whereas not so non-freezable semen may have acceptable freezability and fertility (Dhami and Shelke 2005). Hence periodic detailed andrological investigation is prerequisite to successful breeding programme.

Evaluation of plasma membrane integrity, motility, vigour and morphology of fresh and frozen thawed semen along with the seminal characteristics and their interrelation might establish a basis for the predicting the fertility potential of semen. Hence the objectives of this investigation were to compare seminal attributes between the buffalo bulls of freezable and non-freezable semen and further, establishment of correlation between microscopic evaluation parameters.

MATERIALS AND METHODS

The study was conducted on 12 apparently healthy Murrah buffalo breeding bulls, aged between 5–7 years, maintained at Frozen Semen Bank, RCDF Limited, Bassi, Jaipur. The selected bulls were divided into 2 groups, each comprising of 6 bulls. Group 1 comprised those bulls, which were donating semen of excellent quality with freezable freezability and fertility parameters, whereas, Group 2 included those bulls which were frequently donating either initial non-freezable semen or higher degree of damage during processing (during equilibration or cryopreservation)

Present address: [1]College of Veterinary and Animal Sciences, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh. ✉Corresponding author email: akshays482@gmail.com

but were otherwise healthy. Total 96 ejaculates (8 ejaculates from each of 12 bulls), were collected twice a week by artificial vagina method. The collected semen was subjected to initial examination of volume, colour, concentration and initial motility qualifying after which they were further processed as per standard laboratory procedures. The minimum initial standards were a volume of 1 ml, colour ranging from milky white to creamy, minimum concentration of 500 million/ml. Samples having +3 or more mass movement were considered for further processing in case of freezable ejaculates and below +3 were also considered in case of non-freezable ejaculate evaluation.

The selected ejaculate was diluted with tris egg yolk extender to attain a final concentration of 80 million/ml after which they were filled into French mini straws. These straws were finally shifted to liquid nitrogen containers and stored till use. These were analyzed for percent viability, progressive motility, reaction to 100 mOsmol hypo-osmotic solution (Pant *et al*. 2002) and acrosomal integrity (Watson and Stewart 1979) at four stages of processing namely post-dilution (34°C), post-equilibration (4°C), post-thaw (37°C) and 1 h after incubation at 37°C. Thawing of frozen semen straws for post thaw evaluation was done in water bath at 37°C for 30 sec. The data obtained was analysed using SAS statistical package version 9.2. Multivariate analysis was used to determine the correlations and to frame regression equations and their significance was tested by ANOVA.

RESULTS AND DISCUSSION

Significant differences (P<0.05) were found during the study for various parameters when freezable and non freezable semen was compared (Table 1). Perusal of the Table 2 indicates that sperm viability was significantly (P<0.01) correlated with progressively motile spermatozoa, HOS responsive spermatozoa and acrosomal integrity in freezable and non-freezable ejaculates. Also, progressive motility was significantly (P<0.01) correlated with HOS reactive and acrosomal integrity in freezable and non-freezable ejaculates, respectively (Table 2). Our findings also indicated that HOS reactive spermatozoa percentage was significantly (P<0.01) correlated with acrosomal integrity in freezable and non-freezable ejaculates, respectively (Table 2). The results of the present study were in agreement with some previous work on cattle (Lodhi *et al*. 2008, Sharma *et al*. 2012), human (Jeyendran *et al.* 1984), equine (Mantovani *et al.* 2002), ram (Bohlooli *et al.* 2012) and fresh goat spermatozoa (Fonseca *et al.* 2005).

Various conventional evaluation parameters have been used to assess semen quality. Evaluating a relationship between different semen evaluation parameters is important, as we can judge other parameters on the basis of evaluation of one parameter. El-Sisy *et al*. (2010) showed a significant correlation between live spermatozoa per cent with HOS reactive (r=0.681) and acrosomal abnormality (r=0.220) in buffalo bull spermatozoa which is in agreement to our findings. The percentage of live sperms and acrosome intact sperms has also been shown to be highly correlated with percentage of motile sperms (Kumar 2004, Kirk *et al*. 2005).

Sperm motility is a fairly reliable indication of the viability of fresh and frozen semen (Graham *et al*. 1978). Barnabe *et al*. (1981) associated a higher initial progressive motility to a lower incidence of abnormal acrosomes. El-

Table 1. Comparative functional parameters (Mean±SE) of freezable (n=48) versus non-freezable (n=48) semen during various stages of processing in Murrah buffalo bulls

Parameter	Type of semen	Stage of semen processing			
		Fresh Diluted	Equilibration	0 h Post-thaw	1 h Post-thaw
Live spermatozoa (%)	Freezable	90.88 ± 0.26^{aA} (86–94)	86.44 ± 0.25^{aB} (82–90)	78.29 ± 0.31^{aC} (73–83)	74.00 ± 0.36^{aD} (69–80)
	Non-freezable	64.42 ± 0.91^{bA} (49–79)	48.77 ± 1.11^{bB} (32–63)	29.42 ± 1.10^{bC} (15–44)	10.75 ± 0.78^{bD} (02–25)
Progressive motile spermatozoa (%)	Freezable	86.15 ± 0.34^{aA} (79–90)	73.17 ± 0.38^{aB} (68–80)	63.5 ± 0.29^{aC} (60–68)	40.46 ± 0.50^{aD} (32–49)
	Non-freezable	44.48 ± 0.75^{bA} (30–54)	31.25 ± 0.69^{bB} (20–43)	18.15 ± 0.69^{bC} (08–29)	5.42 ± 0.58^{bD} (00–13)
HOS Reactive Spermatozoa (%)	Freezable	89.19 ± 0.26^{aA} (84–92)	85.27 ± 0.23^{aB} (81–89)	77.05 ± 0.31^{aC} (72–82)	72.90 ± 0.39^{aD} (68–79)
	Non-freezable	63.17 ± 0.93^{bA} (47–77)	47.58 ± 1.09^{bB} (31–61)	28.44 ± 1.09^{bC} (14–43)	9.94 ± 0.79^{bD} (00–24)
Intact acrosome (%)	Freezable	91.21 ± 0.30^{aA} (87–95)	85.48 ± 0.37^{aB} (80–90)	78.77 ± 0.35^{aC} (74–83)	70.79 ± 0.32^{aD} (67–76)
	Non-freezable	65.33 ± 0.88^{bA} (46–80)	49.65 ± 1.04^{bB} (34–61)	32.06 ± 0.81^{bC} (19–43)	15.88 ± 0.64^{bD} (05–24)

^{A,B,C,D}Means with different superscript within rows differ significantly (P<0.05). ^{a,b} Means with different superscript between columns differ significantly (P<0.05); n= Number of ejaculates.

Relationship between parameters		Type of semen coefficient	Correlation Estimate	Regression Equation	Regression
Viability	Motility	Freezable	0.92738**	0.38±0.01	y=57.39+0.38x
		Non-freezable	0.99934**	1.31±0.00	y= 5.76+1.31x
	HOST	Freezable	0.98934**	1.01±0.01	y= 0.43+1.01x
		Non-freezable	0.99934**	1.00±0.00	y= 0.79+1.00x
	Acrosome	Freezable	0.92725**	0.81±0.02	y=16.52+0.81x
		Non-freezable	0.98273**	1.07±0.01	y= –5.43+1.07x
Progressive motility	HOST	Freezable	0.92052**	2.30±0.07	y=–120.32+2.30x
		Non-freezable	0.94212**	0.68±0.02	y=–0.62+0.68x
	Acrosome	Freezable	0.94709**	2.01±0.05	y= –98.40+2.01x
		Non-freezable	0.95266**	0.75±0.02	y= –5.67+0.75x
HOST	Acrosome	Freezable	0.91623**	0.78±0.24	y= 17.38+0.78x
		Non-freezable	0.98272**	1.07±0.01	y= –6.15+1.07x

**(P<0.01).

Sisy *et al*. (2010) reported a significant correlation of motility with live (r= 0.728), HOS reactive (r=0.918) and abnormal acrosome (r=0.277) in buffalo bull spermatozoa. Kumar (2004) reported a significantly positive correlation between sperm motility, live percentage and HOST reactive spermatozoa. Raval and Dhami (2006) also reported a significant (P<0.05) correlation between initial motility with live sperm (r=0.54) which is in concurrence with our findings.

Lodhi *et al*. (2008) reported a positive and significant (P<0.05) correlation between HOST and progressive motility (r=0.649), sperm viability (r=0.880) and morphologically normal spermatozoa (r=0.611) in buffalo bull spermatozoa which is not akin to our findings. The percentage of positive sperms to HOS test varies with bull (Prasad *et al*. 1999), season (Kale *et al*. 2000), mass activity, progressive motility, sperm count and total sperm with intact acrosome (Prasad *et al.* 1999). Comparing of various methods for evaluating sperm plasmalemma of bovine semen, Brito *et al*. (2003) found that the proportion of HOS responsive sperm was only moderately correlated with the proportion of plasmalemma-intact sperm identified by vital stains in contrast to high correlation reported by Correa and Javos (1994).

In conclusion, non-freezable semen was having least viability, progressive motility, reaction to hypo-osmotic solution and acrosomal integrity, so, it can be considered as poor quality semen. As far as correlation between various semen evaluation parameters is concerned, there was a positive correlation for all the parameters in semen of freezable and non-freezable quality.

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